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Authors: Oliver H.P. Burman, Michael T. Mendl

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A novel task to assess mood congruent memory bias in non-human animals

OLIVER H. P. BURMAN<sup>1</sup> & MICHAEL T. MENDL<sup>2</sup>

<sup>1</sup>University of Lincoln, Lincoln, LN6 7DL, UK.

<sup>2</sup>University of Bristol, Bristol, BS40 5DU, UK

Research Paper

Corresponding author: Oliver H. P. Burman

<sup>1</sup>School of Life Sciences,

Joseph Banks Laboratories,

University of Lincoln,

Lincoln LN6 7DL,

U.K.

Email: oburman@lincoln.ac.uk

Tel.: 01522835453

<sup>2</sup>Bristol Veterinary School,

University of Bristol,

Langford House,

Langford,

BS40 5DU,

UK

Highlights:

- We describe a new memory bias task, using social status to alter affective state

- Subordinate rats made errors earlier in tests that followed a negative event
- Dominant rats made errors earlier in tests that followed a positive event
- This new task could be used to assess affective state in a range of animal species

## Abstract

*Background:* Assessing the affective state of animals is important for a range of research areas, including neuroscience. The use of cognitive judgement and attention biases to determine affective state has been demonstrated in animals, but approaches to assess mood-congruent biases in memory have yet to become established. *New Method:* We describe a novel methodology to investigate memory bias in animals, presenting initial data using the influence of social status to manipulate affective state. The method required laboratory rats to achieve criterion at a working-memory task in an eight-arm radial maze before probing their memory of putative negative, positive or neutral events that occurred in specific arms of the maze. They were tested 2hrs and 24hrs after experiencing each event to determine how the affective valence of the event influenced task performance. *Results:* Regardless of social status, rats avoided arms where they had experienced negative events and preferred arms where they had experienced positive events. However, subordinate rats made errors sooner than dominant rats in tests following exposure to the negative event. Furthermore, whilst subordinate individuals made errors earlier in tests following the negative event relative to the neutral or positive event, dominant rats made errors earlier in tests that followed the positive event. *Comparison with existing method(s):* Changes in performance thus appeared to reflect social status and associated affective state, confirming a new method for assessing animal affect. *Conclusions:* This new memory bias task could potentially be used to determine affective state in a range of non-human animal species.

**Keywords:** Emotion; Cognition; Memory; Cognitive Bias; Animal Welfare

ACCEPTED MANUSCRIPT

## 1. Introduction

People in a negative affective state show enhanced attention to negative/threat-related stimuli (an attention bias), may show a memory advantage for negative events (a mood-congruent memory bias) and may interpret ambiguity negatively (a judgement bias), compared to people in a positive affective state who show the opposite bias (Eysenck et al. 1991; MacLeod and McLaughlin 1995; Mogg and Bradley 1998; Bradley et al. 1998; see Paul et al. 2005). Similar cognitive-affective biases (or, simply, 'cognitive biases') in judgement (e.g. rats: Harding et al. 2004; Rygula et al. 2012, 2014; starlings: Bateson and Matheson 2007, dogs: Mendl et al. 2010, and bees: Bateson et al. 2011) and attention (rhesus macaques: Bethell et al. 2012) have now also been observed in a range of non-human animal species experiencing different affective manipulations (e.g. enrichment provision (e.g. Burman et al. 2008a), a genetic animal model of depression (Richter et al. 2012), pharmacological treatment (Rygula et al. 2014)). Measures of cognitive bias may therefore provide a means of assessing the affective state of animals, a central goal in a range of research areas including psychology, pharmacology, neuroscience, and animal welfare science (e.g. File 1995; Dawkins 2000; Panksepp 2003; Mendl and Paul 2004; Rolls 2005).

However, despite increased interest in this area, there has been little focus on developing methods for assessing mood-congruent memory bias in animals. Mood congruent memory bias occurs when subjects are more likely to store or recall information that matches their current mood state (e.g. Bower 1981; Klaassen et al. 2002). Thus, animals in a negative state are predicted to store and recall information about negative events more effectively than animals in a positive state who should be better at memorising positive events.

Demonstration of mood-congruent memory would therefore allow us to infer an animal's affective state, hence providing a new method for assessing animal affect, including positively valenced affective states that are of increasing interest in animal welfare science

but remain difficult to measure (Boissy et al. 2007; Yeates & Main, 2008). Moreover, the enhanced storage and subsequent recall of either positively or negatively valenced memory could have a considerable impact on the welfare of captive animals, bringing both potential benefits and problems (Mendl et al. 2001; Mendl and Paul 2008). For example, calves that had previously received regular positive contact from humans were subsequently less fearful of people and easier to handle (Lensink et al. 2001). Memory biases may partly underlie observed judgement biases, and so their study could reveal potential psychological processes underpinning biased judgements of ambiguity (Mendl et al. 2009). In addition, because there is stronger experimental evidence for memory biases in association with depression rather than anxiety in humans (MacLeod et al. 1986; Mineka and Nugent 1995), studying memory biases may eventually help us to differentiate between similarly valenced affective states (i.e. depression and anxiety) in non-human animal species.

Given the potential value in developing measures of affect-induced memory bias in animals, we sought to extend existing research on cognitive biases to encompass mood congruent memory. In humans, affect-induced memory bias is typically studied by investigating the influence of a subject's affective state on how well they recall verbal stimuli of varying (i.e. positive, negative and neutral) affective valence (e.g. Klaassen et al. 2002). For example, do happier subjects more readily recall positive words? To investigate this in animals requires a non-verbal method. Takatsu-Coleman et al (2013) used a PM-DAT (plus-maze discriminative avoidance task) to assess mood-congruent memory in mice, finding that socially isolated mice showed depressive-like behaviour and enhanced memory for the aversive task.

However, this approach did not include a positively valenced task, potentially restricting its application. To this end, we designed a novel task for rats using the radial arm maze (e.g. Olton and Samuelson 1976; Dubreuil et al. 2003; Brown et al. 2007) in which we could manipulate the valence (i.e. positive, negative and neutral) of events occurring in specific

arms and study whether, and how, memories of these events impacted upon subsequent task performance.

We hypothesised that memory of affectively valenced events should influence subsequent performance in the task as follows: (1) the locations of negative events should be avoided and locations of positive events preferred indicating that rats can update the value of specific locations in a complex environment; (2) if mood-congruent memory is operating, animals in a negative state should be better at remembering, and hence avoiding, the negative location, whilst those in a positive state should be better at remembering the positive location; (3) mood-congruent memories may occupy attention and thereby exert a pro-active interference effect on task performance (Mendl et al. 2001; Woodson et al. 2003) such that animals in a negative state make more errors when retrieving negatively valenced information than those in a positive state.

To evaluate the potential for this new task to discriminate between subjects experiencing different affective states, we decided to manipulate affect in our experimental subjects by using existing variation in social status. Subordinate animals in social groupings are often exposed to long-term social stress, and are therefore likely to experience chronic negative affective states such as depression (Sapolsky 2005; Arregi et al. 2006). A number of lines of evidence support this assertion, including behavioural observations (e.g. subordinate individuals sleep less: Hurst et al. 1996), physiological differences (e.g. elevated basal glucocorticoid levels in subordinates: see Sapolsky 2005, although see Tamashiro et al. 2005), and variation in immune susceptibility (e.g. impaired immune response to challenge in subordinates: Raab et al. 1986). As a consequence, dominant-subordinate social relationships in animals are commonly used to model affective disorders, particularly depression (e.g. Malatynska and Knapp 2005; Arregi et al. 2006), and research into the affective states that underpin these relationships may allow us to refine our use of such



models and increase our understanding of how to improve the welfare of socially-housed captive animals. We thus tested our hypotheses using subordinate rats (in a putatively more negative state) and dominant rats (in a putatively more positive state).

## 2. Materials and Methods

### 2.1 Subjects

We used fourteen male Lister-hooded rats (Harlan, UK), approximately 12 months old at the start of testing. The rats had been used (at least nine months previously) in a study of incentive contrast (Burman et al. 2008b). They were housed in pairs in standard cages (33cmx50cmx21cm) on a 12hr reversed light cycle, lights off 0800-2000, with food (Harlan Teklad Laboratory Diet) and water available ad libitum. The housing room was lit with a 60W (380 lm) red light bulb allowing the researcher to see the rats during lights off. Rats could be individually identified by natural variation in their coat markings. All the cages were provided with identical enrichment items (e.g. a shelter, an aspen wood gnaw block). This study was carried out under UK Home Office licence (PPL 30/2249) and complied with ARRIVE guidelines.

### 2.2 Treatment: *determining social status*

Relative social status in laboratory rats can be determined by observing spontaneous agonistic behaviour within a social group (e.g. Blanchard et al. 1991; Militzer 1995; Hurst et al. 1996), via social defeat and resident/intruder paradigms (see Martinez et al. 1998) or by using resource competition tests (e.g. Gentsch et al. 1990; Militzer 1995; Millard and Gentsch 2006). We used a combination of all three approaches, by observing agonistic behaviour in long-established social groups (pairs) – with their implication of inescapable repeated social defeat over a long period of time – and competition for two different food-based resources. All seven pairs of rats (N=14) had been housed in the same pairs for at least nine months, and were monitored to reveal which of the two rats was (relatively) dominant

and which subordinate. This was determined by carrying out two different food competition tests, one a competition for a piece of apple (12 tests per pair), the other a competition for a sugar-coated cereal (e.g. Mason et al. 2004) (18 tests per pair), and observing spontaneous agonistic behaviour (behavioural observations carried out for one hour/day for 12 days for each pair). Categorisation as 'dominant' required that one rat of each pair emerged as a consistent 'winner' (i.e. it obtained the food in the competition test(s) and/or avoided social defeat) for at least two of the three approaches to social status assessment.

### *2.3 Apparatus*

In a different room to that in which the rats were housed, we positioned an eight arm radial maze (arm length 70cm, arm width 10cm, hub diameter and height 30cm, Panlab) made of black Perspex with manually operated guillotine doors leading from the decision area/central maze hub to each arm. We carried out trials during the dark phase of the reversed light cycle to minimise disruption (Abou-Ismaïl et al. 2008), with dim white lighting (25W, centrally positioned above the maze) to allow subjects and researcher to see the apparatus. The whole maze was raised 100cm above the ground. There were recessed goal pots (width 4cm, depth 3cm) located 6cm from the end of each arm. Both internal cues (cartoon animal stickers: positioned above the entrance to each arm) and external cues (numbers in a large font size: attached to face the end of each arm) were provided for the rat as an aide to spatial memory. The guillotine doors were operated using a pulley system from behind a screen so that the researcher was not visible to the subjects. Also behind the screen were a video and monitor linked to an overhead video camera allowing the subjects to be observed, and their behaviour recorded, remotely. Maze arms were cleaned with a 70% ethanol solution before each rat was trained/tested.

### *2.4 Procedure*

Habituation: Before habituation to the radial arm maze, we gave the rats prior exposure to the food used as a reward in the task (Noyes Dustless Precision Pellets, 45mg, Bio-Serv, UK), placing six pellets in each cage of rats for three consecutive days. Rats were then habituated to the apparatus on four occasions (one per day) in order to ensure familiarity with the test conditions, including: obtaining food in the apparatus; being enclosed in the maze centre; and exposure to all eight maze arms. For the habituation sessions 1-3, we randomly scattered five pellets in the centre of the maze and five pellets in each of the eight arms. Individual rats were placed gently into the central maze hub with all guillotine doors closed for 20seconds, we then opened all the doors and kept them open until 5mins had elapsed. We recorded the number of pellets eaten and the number of arms visited (defined as a rat reaching the end of the arm). For the final habituation session, pellets were only scattered in the arms of the maze. Training then began, with rats by now comfortable at being picked up and placed on the maze, exploring with little (if any) defecation or urination, eating most/all of the pellets and visiting most/all of the maze arms. The rats were not food restricted prior to training/testing.

Training: During training, pellets were placed only in the goal pots at the end of each arm, one in each pot. From the first training session onwards, the rats were enclosed in the maze centre between each arm visit for 10sec, in order to prevent them learning to use non-memory searching strategies to complete the radial arm maze (RAM) task (Dubreuil et al. 2003), as well as being closed in the centre for 20sec at the start of each session. Rats received one session per day in a different order, balanced for treatment, on each day. Twenty seconds after placing the rat into the maze centre, the researcher raised all doors simultaneously allowing the rat to enter an arm of its choice. Arm entry was classified as when all four feet entered an arm. Once an arm had been chosen, the doors to all other arms were closed. We recorded which arm was visited, in what order, the number of errors (revisits) and the number of arms visited before the first error (eight if no errors made) (e.g. Dubreuil et al. 2003). The session

continued until either: 1) all eight arms had been visited; 2) the rat had made 16 visits (including revisits); (3) 15min had elapsed. If all eight arms had been visited, then the total time taken to complete the task was noted as an estimate of motivation and locomotor activity (e.g. Ohl and Fuchs 1999). Training continued until three consecutive sessions were completed with a maximum of 3 errors in total, and no more than 2 errors in any individual session, e.g. 2, 0, 1. The time taken to reach criterion was compared between dominant and subordinate rats.

Testing: Once each rat reached criterion it experienced an ‘event’ (either positive ‘+’, negative ‘-’ or neutral ‘0’) and was then tested two hours and 24hrs later to see how well that particular event was remembered, and to what extent it influenced subsequent performance. Rats were exposed to all three events, one at a time, according to a Latin Square design, e.g. rat 1: 0,+,-; rat 2: +,-,0; rat 3: -,0,+, such that any potential order effect was taken into account. Rats were randomly allocated to order sequences. Exposure to an event consisted of taking the rat from its home cage to the RAM and giving it a single forced trial in which only one arm was available for the rat to enter. The goal pot of the arm contained food pellets that defined the nature of the event as follows: ‘neutral’ (one pellet – as for training); ‘positive’ (12 pellets (a large amount previously shown to be strongly preferred over a single pellet by rats (Burman et al. 2008b); ‘negative’ (12 quinine soaked pellets (previously shown to be aversive to rats (Burman et al. 2009)). Emotions can be defined as states elicited by rewards (something for which an animal will work) and punishers (something that an animal will work to avoid) (Rolls 2005), and so the experience of the negative and positive events was anticipated to elicit contrasting affective states in the subjects. Once the rat returned from the ‘forced’ arm to the centre of the maze, it was returned to its home cage. Two hours and 24hrs after experiencing an event, rats were tested in the RAM, as for training, with all eight arms available and each arm baited with a single pellet, with the same measures recorded.

In testing, we wanted to see whether or not the rats avoided or preferred the arm in which they had previously experienced the events (positive event: approach; negative event: avoid; neutral event: random), how their subsequent performance (i.e. accuracy) was affected, and

whether either of these measures was influenced by treatment (i.e. social status) indicating a mood congruent memory bias. By testing on two occasions, the first time two hours after the forced trial (and experience of an event), the second time 24hrs after the forced trial, we could investigate the duration of any effects on performance. Testing required two consecutive days (to incorporate tests at both two hours and 24hrs after each event exposure) for each of the three events. There was at least 48hrs separating the rats' experience of each event.

Arm allocation for events: In order to avoid any pre-existing preferences for particular arms influencing the order in which arms were chosen during testing, the allocation of the maze arms in which the rats experienced the different events was not random. Instead, arms were selected based on the previous choices of each individual rat. We calculated the average 'preferred' arm (the earliest arm, on average, to be visited), the 'least preferred' arm (the last arm, on average, to be visited) and the 'intermediate' arm (the fourth arm, on average, to be visited) using the order in which the arms were visited in the same three consecutive trials during which the rats achieved criterion during training. We then used the 'preferred' arm for the negative event because this provided the greatest opportunity to show avoidance behaviour (i.e. if the least preferred arm were used this would likely be visited late on in the following test, and we would not be able to tell if this was due to a strong memory of the negative event or an intrinsic order preference for visiting this arm late in the sequence). Similarly, we used the least preferred arm for the positive event (i.e. providing the most opportunity to show increased approach behaviour), and the intermediate arm for the neutral event.

## 2.5 Data analysis

We used parametric statistical tests (e.g. General Linear Models (GLMs), unpaired t-tests) unless the data failed to meet the requirements for parametric tests (e.g. normality), in which case the data were analysed using appropriate non-parametric tests (e.g. Chi-square, Mann-Whitney). The specific statistical tests and models used are noted at the appropriate points in the Results. The statistics package used was SPSS version 21.

### 3. Results

#### 3.1 Dominance relationships

Our criterion for a clear dominance relationship was that one rat of each pair ‘won’ a minimum of 10/12 wins in the ‘apple’ tests (83%, Chi-square:  $\chi^2=5.3$ ,  $P=0.021$ , Cohen’s  $d=1.8$ ) and/or 14/18 in the ‘cereal’ tests (78%, Chi-square:  $\chi^2=5.6$ ,  $P=0.018$ , Cohen’s  $d=1.3$ ). For spontaneous agonistic behaviour, because it was rarely seen, percentages of  $\geq 80\%$  were used to indicate a clear dominance relationship. The criterion for a consistent dominance relationship was that one rat of each pair emerged as a clear ‘winner’ for at least two of the three types of social status assessment. Using the aforementioned criteria, seven out of the eleven cages that we observed showed a clear and consistent dominance relationship, and only those seven cages (14 animals) were selected for continued use in the experiment. Inevitably, because the animals were pair-housed, dominance relationships of each individual were only relative to its own cage-mate.

#### 3.2 Training

The following comparisons were made using only Training days 1-11 (inclusive) with all 14 rats included, because after this point individual rats began to reach criterion and move on to testing. Comparisons were made for each of three task performance variables (total time, no. errors, no. arms before first error), with Time (1-11: within-subjects) and Treatment (‘Dominant’/‘Subordinate’: between-subjects) as factors using a repeated measures General Linear Model (GLM).

Total time: Those rats that failed to complete the session within 15min were allocated default values of 900sec. Whilst there was no significant effect of Treatment ( $F_{1,12}=0.203$ ,  $P=0.66$ ,  $\eta^2_p=0.017$ ) or a Treatment\*Time interaction ( $F_{10,120}=1.36$ ,  $P=0.208$ ,  $\eta^2_p=0.102$ ), there was a highly significant effect of Time ( $F_{10,120}=4.56$ ,  $P<0.001$ ,  $\eta^2_p=0.275$ ). A post-hoc Tukey

pairwise comparison revealed that all rats were quick to complete the first session, then slowed down (slowest on Day four), before gradually speeding up after that.

No. errors: Rats that failed to complete the task either within 15mins or by 16 arm visits, were allocated a default value of eight errors (maximum no. errors observed). We found no significant treatment effects, either individually ( $F_{1,12}=0.004$ ,  $P=0.949$ ,  $\eta^2_p<0.001$ ), or in interaction ( $F_{10,120}=1.08$ ,  $P=0.38$ ,  $\eta^2_p=0.083$ ), but there was a significant effect of Time ( $F_{10,120}=4.27$ ,  $P<0.001$ ,  $\eta^2_p=0.262$ ), with initially (Day one) a low number of errors, increasing up to a peak (highest on Day four), before a gradual reduction in errors up to Day 11 (see Figure One).

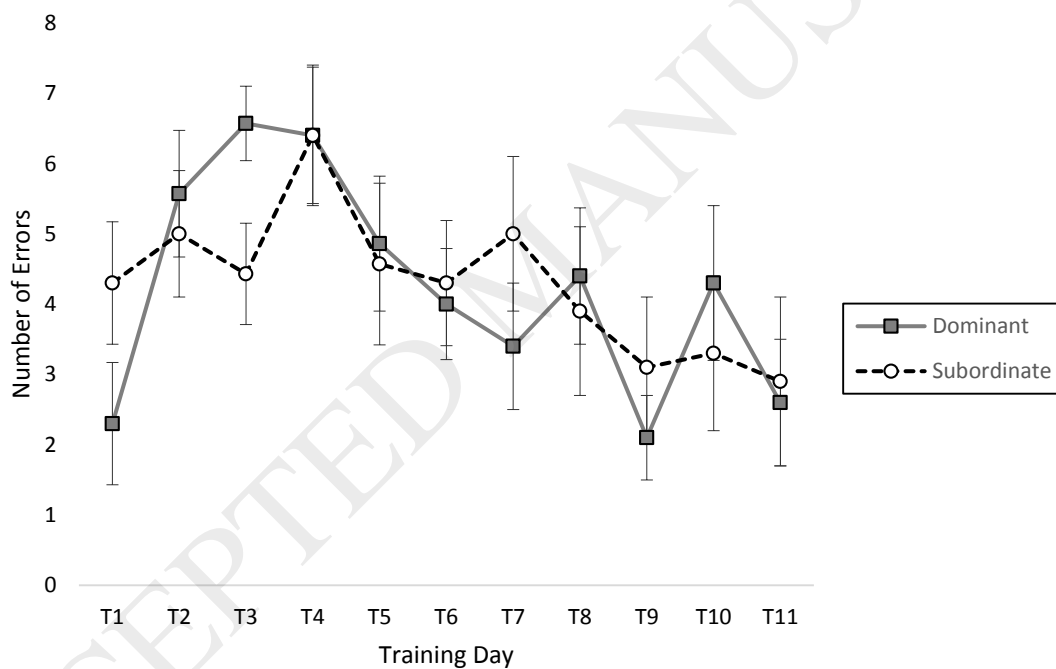


Figure One: A graph showing the number of errors (mean  $\pm$  st.error) made by rats for Training days 1-11 (N=14).

No. of arms visited before first error: There was no significant Treatment effect ( $F_{1,12}=0.02$ ,  $P=0.969$ ,  $\eta^2_p<0.001$ ) or interaction ( $F_{10,120}=0.696$ ,  $P=0.727$ ,  $\eta^2_p=0.055$ ), but a significant effect

of Time ( $F_{10,120}=2.244$ ,  $P=0.019$ ,  $\eta^2_p=0.158$ ), with a gradual increase over time in the number of arms visited before the first error was made.

A total of nine animals, five 'subordinate' and four 'dominant', had reached criterion by (and including) Day 22, and, due to timing constraints preventing further training taking place, only those nine individuals went on to be tested. The subsequent analyses are therefore based on those nine animals that successfully achieved criterion. There was no difference between the Treatments in the number of trials required to reach criterion (Mann-Whitney:  $U=9$ ,  $N=9$ ,  $P=0.805$ , Cohen's  $d=0.112$ ), nor was there any difference in the total number of animals from each treatment to reach criterion (Chi-square:  $\chi^2_{1,9}=0.111$ ,  $P=0.739$ , Cohen's  $d=0.2234$ ).

### 3.3 Testing

Adjusted arm visit order: Because we had selected the location of the arm in which each rat experienced each type of event based on its previous preference (See 'Arm allocation for events'), we examined the difference between the order (1<sup>st</sup> – 8<sup>th</sup>) in which 'event' arms were visited in the test (*actual choice order*) and the average order in which those arms had been visited previously (and which was the basis for choosing the location of a specific event – *average choice order*). We thus calculated a new variable 'adjusted arm visit order' by subtracting the *actual* choice order from the *average* choice order for each event. Using a repeated measures GLM, we found no significant effects of either Treatment ('dominant'/'subordinate': between-subjects), Time (2hrs/24hrs: within-subjects) or their interactions (all  $P>0.1$ ), but a highly significant effect of Event (negative/positive/neutral: within-subjects) ( $F_{2,14}=14.072$ ,  $P<0.001$ ,  $\eta^2_p=0.668$ ). A post-hoc (Tukey pairwise comparison) analysis revealed significant differences, or strong trends for differences, between each event (negative/positive  $P=0.001$ , negative/neutral  $P=0.013$ , positive/neutral  $P=0.069$ ) indicating that, regardless of either Treatment or Time, rats visited arms where they had



previously experienced 'negative' events,  $P=0.013$  average, arms where they had previously experienced 'positive' events,  $P=0.069$  average, and showed no difference to the average order of visits to arms where they experienced 'neutral' events (see Figure Two).

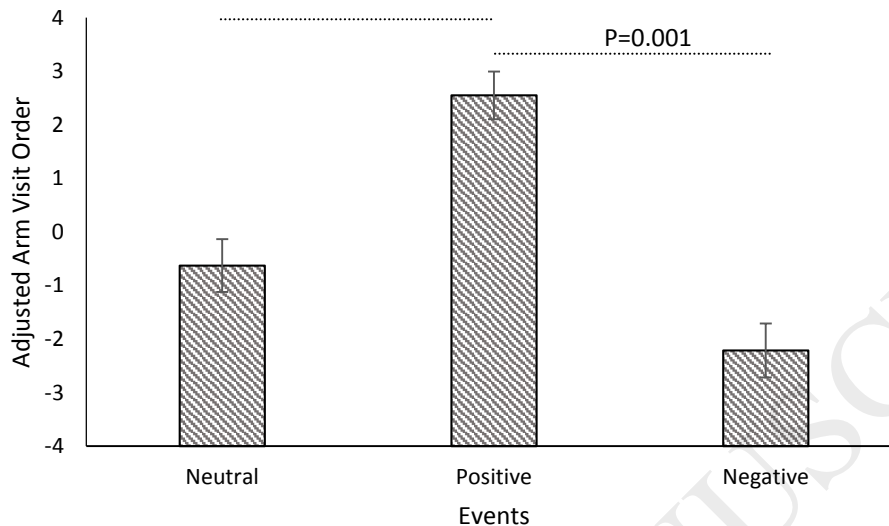


Figure Two: A graph showing the adjusted arm visit order (mean  $\pm$  st.error) for the neutral, positive and negative events. Data are pooled for Treatment and Time.

No. of arms visited before first error: We looked to see if there was any significant effect of Time, Event and/or Treatment on the number of arms visited before the first error was made. There was a significant Time\*Event\*Treatment interaction ( $F_{2,14}=4.145$ ,  $P=0.039$ ,  $\eta^2_p=0.372$ ). Post-hoc (independent t-tests) comparison of this interaction revealed that there were no significant differences between treatments 24hrs after exposure to events, but 2hrs after exposure to the negative event 'subordinate' rats made their first error significantly earlier than 'dominant' individuals ( $t=-2.898$ , d.f.=7,  $P=0.023$ , Cohen's  $d=-2.204$ ), whereas we found no such treatment differences following exposure to either positive or neutral events (both  $P>0.1$ ) (see Figure Three).

(a)

<InlinelImage3>

(b)

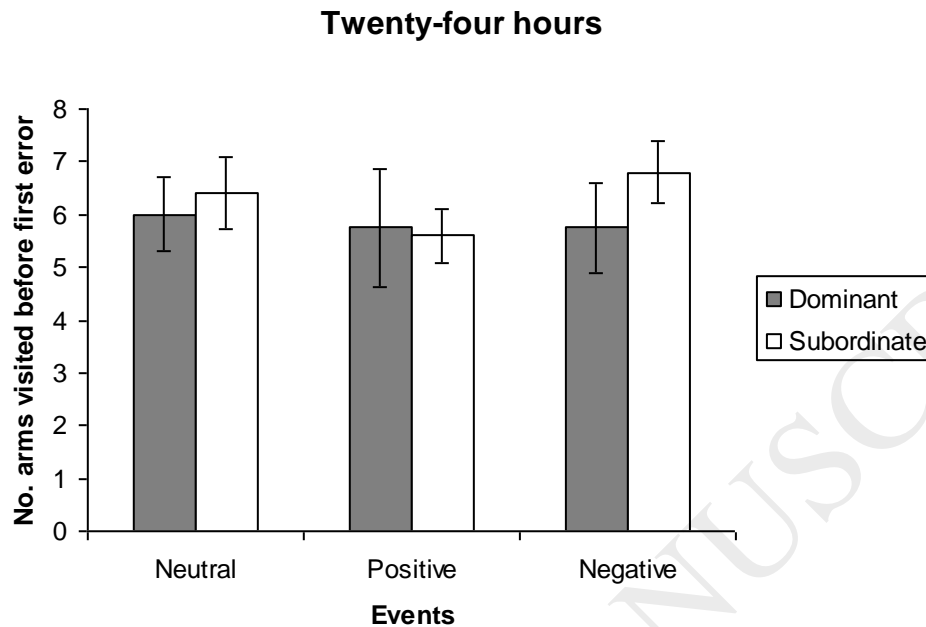


Figure Three: Graphs showing the no. of arms visited before the first error (mean  $\pm$  st.error) for the subordinate and dominant animals (a) two hours and (b) twenty four hours after experiencing the neutral, positive and negative events. \*  $P < 0.05$

As part of the post-hoc investigation of the significant three-way interaction, we also compared within treatments using repeated measures GLMs, to see if there were differences in the way in which either 'subordinates' or 'dominants' responded to the different events at the two different time intervals. For 'subordinates' we found a significant difference between the three types of event ( $F_{2,8}=7.43$ ,  $P=0.015$ ,  $\eta^2_p=0.65$ ) after two hours, but not after 24hrs ( $P>0.1$ ). Post-hoc comparison revealed significant differences between the negative and the other two events (neutral/negative:  $P=0.047$ ; positive/negative:  $P=0.037$ ; neutral/positive:  $P>0.1$ ), i.e. subordinate individuals made their first error earlier following the negative event than after experiencing either of the other events (Fig. 2a). For 'dominants' there was a close to significant difference between the events after two hours

( $F_{2,6}=4.88$ ,  $P=0.055$ ,  $\eta^2_p=0.619$ ) but not after 24hrs ( $P>0.1$ ). Post-hoc investigation of the trend revealed a significant difference between the negative and positive events (positive/negative:  $P=0.016$ ; positive/neutral & neutral/negative:  $P>0.1$ ), i.e. dominant individuals made their first error earlier following the positive event than after experiencing the negative or neutral events (Fig. 2a).

No. errors: There were no significant main factor effects or interactions (all  $P>0.1$ ) for the total number of errors made during testing.

Total time: There were no significant main factor effects or interactions (all  $P>0.1$ ) for the total time taken to complete the task during testing.

#### 4. Discussion

Despite the increase in use of cognitive bias as an approach to assess affective state and hence welfare (Mendl et al. 2009, Gygax 2014, Baciadonna & McElligott 2015), the predominant focus has been on judgement, and to a lesser extent, attention bias (see Introduction) and there is clearly a need to develop approaches to assess memory bias. The method we have developed here offers potential as a new measure of mood-congruent memory, and employs readily available apparatus and widely used training procedures. Our preliminary data indicate that performance in the task may be influenced by affective state, although further confirmatory evidence is needed, ideally from a range of different affective manipulations that might influence long-term affective state (e.g. environmental enrichment, social defeat).

We hypothesised that if subjects had enhanced memories for the affectively valenced events that they had experienced, then this would be reflected in the order in which maze arm visits were chosen and/or performance accuracy – and that this influence on behaviour would be mood congruent. For arm choice order, all subjects, regardless of either social

status or time of testing (i.e. 2hrs/24hrs), visited arms in which they had previously experienced negative events later than their average order of choice for those arms, visited arms in which they had experienced positive events earlier than average, but showed no change in arm visit order relative to average following the experience of a neutral event. This demonstrates that subjects could update the value of different locations in a relatively complex environment, thus allowing the task to be used to assess mood-congruent memory. However, our prediction that 'subordinate' animals would have enhanced memory for negative events and hence show a stronger avoidance of the location of these events compared to 'dominant' individuals (and vice versa) was not supported by measuring order of arm choice. This contrasts with Takatsu-Coleman et al (2013) who found a mood-congruent memory for a negatively valenced task in mice following 12 hours of social isolation. It is possible that our absence of mood congruence for this measure was due to ceiling and floor effects resulting from the relatively small number of arms that were available, and that use of a 12-arm radial maze might increase the chance of finding this effect. Although, the small sample size used in this preliminary study means that caution should be taken in the interpretation of the results, particularly those that are not statistically significant, until further exploration of this methodological advance has been carried out.

However, in terms of performance accuracy, our affective manipulation did appear to influence the number of arms visited before the first error was made. Subordinates made errors earlier than dominants following exposure to a negative event, but not after exposure to either a positive or neutral event. Although we did not observe the opposite effect of dominant individuals making errors earlier than subordinates following exposure to a positive event, we did find that subordinates made errors earlier in tests which followed a negative event compared to those which followed neutral or positive events, whereas dominant rats made errors earlier in tests that followed a positive event. These findings

suggest that the performance of subordinates - individuals more likely to be in a negative affective state - was most impaired following the experience of a negative event, and that performance of dominants - individuals likely to be in a relatively positive state - may be more impaired by positive events. One potential explanation for these findings is that the memory of a mood congruent event shifts attention away from performance of the task at hand, so decreasing effective task performance (e.g. Metcalfe et al. 1987). Attention during task performance may be proactively interfered with by particularly salient information already held in the memory (e.g. memory of negative events in animals in a negative affective state and memory of positive events for animals in a positive state) - possibly because attention to affectively laden events disrupts information storage during the working memory task (e.g. Mendl et al. 2001). For example, predator exposure was observed to impair spatial working memory (i.e. 'within-trial' errors) without adversely affecting spatial reference memory (i.e. 'between-trial' errors) in rats (Woodson et al. 2003).

When implementing a manipulation of affective state, it is important that the task used allows us to identify any potential effects on general learning and memory ability. In this case, we found no significant effect of social status on measures of performance during the first eleven days of training for all 14 rats, and there was also no difference in the time taken to reach criterion or the total number of individuals that achieved criterion. Taken together, the results suggest that there was no general difference in overall learning/memory abilities between dominant and subordinate animals - either for all 14 rats or just for those nine rats that achieved the training criterion. This lack of a mood-congruent effect during training could be because the initial emotional salience of the outcomes was reduced with repeated experience. Whereas during testing, when there was a sudden, unexpected, change in outcome, this induced a significant emotional salience for that specific event relative to expectation (e.g. a positive/negative contrast effect) which could then be influenced by affective state (e.g. Burman et al. 2008b).

Like those tasks designed to assess judgement bias (e.g. Jones et al. 2017), our proposed memory bias task requires training of the subjects prior to testing. This not only increases the input required by the researcher, it can add an 'unknown' element to the duration of the task because testing itself can only start once animals achieve criterion. In this study, after 22 days of training, only 9/14 rats (c.64%) had achieved criterion - although the overall improvement in performance (of all 14 rats) during training suggested that the remaining rats were approaching criterion. Given that testing itself took an additional 10 days (two days for each 'event' with 48hrs between each of the three 'events') the total time taken to assess one individual (32 days) took longer than judgement bias tasks using spatial cues in rats (e.g. 9 days, Burman et al. 2008a) or those using tactile cues (e.g. 4 days, Brydges & Hall, 2017). Whilst this might limit the task's feasibility in its current form, it is likely that the method could be refined to speed up both the training and testing phases. For instance, given that most statistically significant results were observed during testing 2hrs after 'event' exposure, this suggests that testing after 24hrs could be abandoned, speeding things up considerably. Training could also be faster and more effective if a degree of food restriction were implemented to increase task motivation. Further refinement of the approach is therefore still possible, including the use of additional response measures (e.g. latency to enter the arms) and, as previously mentioned, it is important to investigate a range of different long-term affective manipulations, many of which (e.g. environmental enrichment) could be studied without task modification - but the preliminary data reported here demonstrate the potential of this approach.

#### *4.1 Conclusion:*

We have developed and described a novel task to assess the presence of a mood-congruent memory bias in animals that could potentially be adapted to determine affective state in a range of species - a central goal in psychological, pharmacological, and animal welfare

research. The results provide preliminary evidence of a mood congruent memory bias in a non-human animal species as previously identified in humans (e.g. Denny and Hunt 1992; Bradley et al. 1996; Klaassen et al. 2002), and this approach offers an opportunity to extend the existing range of cognitive bias research in animals.

Conflicts of interest: The authors declare that they have no conflicts of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This work was carried out under UK Home Office licence (PPL 30/2249).

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Compliance with ethical standards

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